Rapid formation of acrylated microstructures by microwave-induced thermal crosslinking

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14. ABSTRACT

We present a rapid and highly efficient method to form microstructure of poly(ethylene glycol) (PEG)-based acrylates by microwave-induced thermal crosslinking. PEG-based polymeric microstructures such as polymer microarrays and microwells were fabricated on 3-(trimethoxysilyl)propyl methacrylate (TMSPMA)-coated glass slides that were placed on top of a silicon wafer. In comparison to ultraviolet (UV) irradiation curing, microwave-induced thermal crosslinking could be completed within 10s without thermal degradation or oxygen inhibition in the presence of ambient oxygen. Furthermore, the activation of surviving free radical impurities by microwave-induced heating enabled crosslinking even without an exogenous radical initiator (e.g., 2,2?-azobisisobutyronitrile (AIBN)). This approach can be beneficial for fabricating various PEG-based microstructures for high-throughput screening assays, cellbased biosensors, and biomedical microdevices.

15. SUBJECT TERMS

microwave-induced heating; ultraviolet (UV) irradiation curing; polymer microarray; poly(ethylene) glycol diacrylate (PEGDA); ratio of projected area (RPA)

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Introduction

Polymeric microstructures play an important role in various biomedical applications such as drug delivery,[1] tissue engineering,[2] and cell-based high-throughput screening.[3] In particular, poly(ethylene glycol) (PEG)-based polymers have been widely used for synthesis of various polymeric microstructures due to their resistance to protein and cell adhesion, nontoxicity, nonimmunogenicity, and blood compatibility.[4] In most cases, structurally diverse PEG-based acrylates have been crosslinked by using ultraviolet (UV)-irradiated free radical polymerization processes.[5] Free radical photopolymerization for curing PEG-based acrylates in air is often influenced by oxygen inhibition,[6] which causes undercuring, tacky surface properties, short polymer kinetic chain length, and slow polymerization rates.[7] To overcome the oxygen inhibition, there have been attempts to consume oxygen by using high photoinitiator concentrations and light intensities as well as by using reactors containing inert gases,[8] however, efficient reduction of oxygen inhibition still remains a challenge.

An alternative to photocrosslinking is controlled/living radical polymerizations including atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP), and reversible addition-fragmentation chain transfer (RAFT) polymerization.[9] While these controlled mechanisms have recently attracted attention of many research groups, there is concern about the drawbacks of these technologies such as metal impurities and slow polymerization rates.

Microwave heating has been previously used in drug discovery,[10] organic and polymer chemistry,[11] microfluidic[12] and tissue engineering. In particular, microwave-assisted free radical polymerization has been widely exploited to accelerate free radical polymerization process.[13] Main advantages of this approach are short reaction times, high yields, and less side reactions. Despite these advantages, use of microwave heating has not been adapted for patterning of polymers in comparison to other methods such as nitrogen-blanketed UV-irradiated polymerization,[8] redox-initiated polymerization[14] and

plasma polymerization.[15] Therefore, the development of technique with reduction of oxygen inhibition and rapid crosslinking may be beneficial in the synthesis of micropatterned polymeric structures.

In the early studies, household domestic microwave ovens were often used for organic synthesis using solvent. Although research grade microwave reactors are more reliable and reproducible, modified household microwave ovens are still used in various laboratories for free radical polymerizations due to the relative high cost of the microwave reactor. To overcome the limitations of using household microwave ovens, solvent-free systems[16] have been used for microwave-assisted organic synthesis to enable a clean, efficient and economical reaction.[17] Moreover, dry reaction technique avoids solvents with low boiling points making the use of a household microwave oven safe.[18] Furthermore, since the microwave irradiation activates a semiconductor,[19] it can be used to rapidly heat a silicon wafer up to 1000°C (125°C/second) to induce rapidly crosslinking of the polymer.[20]

Results and Discussion

In this paper, we developed an approach to crosslink acrylated polymers to generate microstructures by using microwave-induced heating. This was achieved by using the scheme shown in Figure 1. Crosslinked poly(ethylene glycol)$_4$ diacrylate (PEG_4DA) microspots were fabricated on 3-(trimethoxysilyl)propyl methacrylate (TMSPMA)-coated glass slides that were placed on top of a silicon wafer by using microwave-induced thermal crosslinking with 1% 2,2´-azobisisobutyronitrile (AIBN) for 10s in the presence of ambient oxygen (Figure 1 and see supporting information (S.I.) experimental part). Microscopic and scanning electron microscope (SEM) images show that microwave-induced heating can form homogeneous microspots (Figure 1b and see S.I. Figure S1). To enhance the energy transfer from microwaves to the acrylated monomers, the substrate was placed on a silicon wafer. As shown in microwave-induced heating profiles in Figure 2a, glass slides on the top of the silicon wafer were heated up to 204°C within 10 s in comparison to glass slides (38°C in 10 s) without the silicon wafer. The glass slide on silicon wafer became warmer than the neat silicon wafer due to a

loss of heat for neat silicon wafer in air during measurement of heat. Thus, as the microwave irradiation rapidly heats the silicon wafer,[20] heat is transferred from silicon wafer to glass slide. Moreover, a rotating plate of a microwave oven allowed samples to heat in a uniform and homogeneous fashion. During microwave-induced heating, PEG_4DA crosslinking was observed between 7 s (139°C) and 10 s (204°C). In contrast, the acrylated precursors on glass slides without the silicon wafer were not completely polymerized presumably due to lower temperatures. Interestingly, the time required to crosslink PEG_4DA using conventional thermal heating (190°C) was longer (30 s) (see S.I. Figure S2), suggesting that heat transfer may be enhanced in microwave-induced heating.

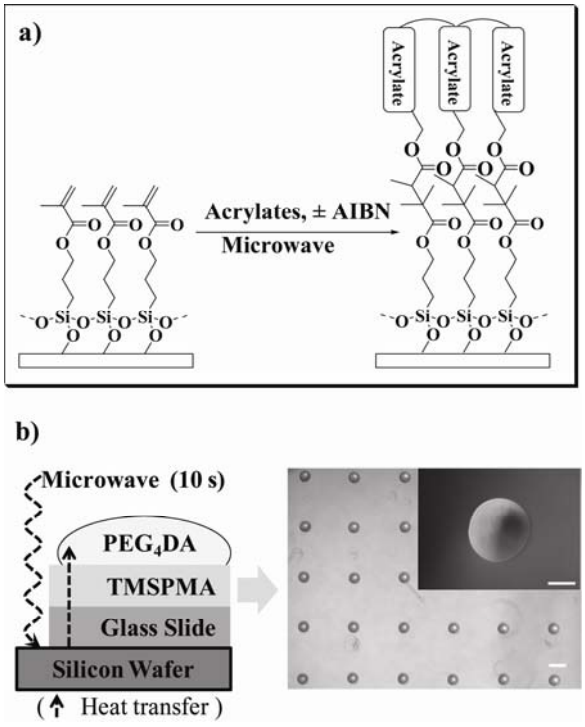

Figure 1. Polymerization of acrylate by using microwave-induced heating. (a) Crosslinking of acrylate by microwave-induced heating. (b) Scheme of microwave-induced heating technique and phase contrast image of a crosslinked PEG_4DA after microwave-induced heating with 1% AIBN for 10s in the presence of ambient oxygen. Scale bars are 100 µm (top inset) and 300 µm.

As compared to the microwave-induced crosslinking technique, polymerization of PEG_4DA containing 1% (w/v) 2,2-dimethoxy-2-phenyl acetophenone (DPA) photoinitiator was incomplete under UV irradiation (20 mW/cm^2) even after 600 s of exposure compared to that of the microwave-induced heating technique. This may be due to the oxygen inhibition effect (see S.I. Figure S3) that oxygen retards the polymerization reaction as oxygen reacts with the free radicals that propagate the reaction.[6] Therefore, it may be that the microwave-induced heating can generate significantly more free radicals that overcome the oxygen inhibitory effect. This may also eliminate the need for inert gases (e.g., argon and nitrogen gases) and increase the reaction speeds.

To assess the chemical properties of the crosslinked microstructures, the Fourier transform infrared spectroscopy (FTIR) spectra of microwave- and UV-irradiated crosslinked polymers were analyzed. As shown in Figure 2b, FTIR spectrum of uncrosslinked PEG_4DA monomer (Figure 2b (i)) before microwave-induced heating was significantly different than the crosslinked PEG_4DA macromer structures (Figure 2b (ii)). The spectrum of the uncrosslinked PEG_4DA monomer shows acrylic vinyl group peaks at 1619 cm^{-1} (C=C), 1407 cm^{-1} (=CH_2), and 984 cm^{-1} (Figure 2b (i)).[21] These characteristic vinyl group peaks disappear in the spectra of the crosslinked PEG_4DA (Figure 2b (ii)). Moreover, shift of carbonyl group from 1720 cm^{-1} (C=O of monomer) to 1727 cm^{-1} (C=O of polymer) is shown in Figure 2b (i) and 2b (ii), respectively. The disappearance of vinyl group and shift of carbonyl group implied that PEG_4DA monomers are crosslinked by using microwave-induced heating with 1% AIBN for 9 s in the presence of ambient oxygen. Similarly, as shown in Figure 2b (iii), the spectrum of the PEG_4DA crosslinked by using microwave-induced heating without 1% AIBN does not also have the characteristic peaks. Both of the crosslinked polymers' spectra show an ester functional group at 1724 cm^{-1} (C=O stretching), 1248 cm^{-1} (C-O stretching), 1162 cm^{-1} (C-C stretching) and an ether functional group between 1096 and 1046 cm^{-1} (C-O-C asymmetric and C-O-C symmetric stretching).[22] These bands strongly indicate that the microwave-induced polymerization of PEG_4DA monomer took place

under ambient oxygen without thermal degradation of ester and ether functionalities. The spectrum of the PEG_4DA crosslinked with 1% DPA by using UV irradiation curing under nitrogen atmosphere for 60 s is shown in Figure 2b (iv), further supporting that microwave-induced heating could be used to crosslink PEG_4DA polymer under ambient oxygen. However, same UV-irradiated polymerization was incomplete under ambient oxygen for 600 s (Figure 2b (v)). Therefore, in comparison to UV-induced crosslinking, the crosslinking of PEG_4DA monomer in the presence of ambient oxygen by using the microwave-induced heating technique is highly efficient.

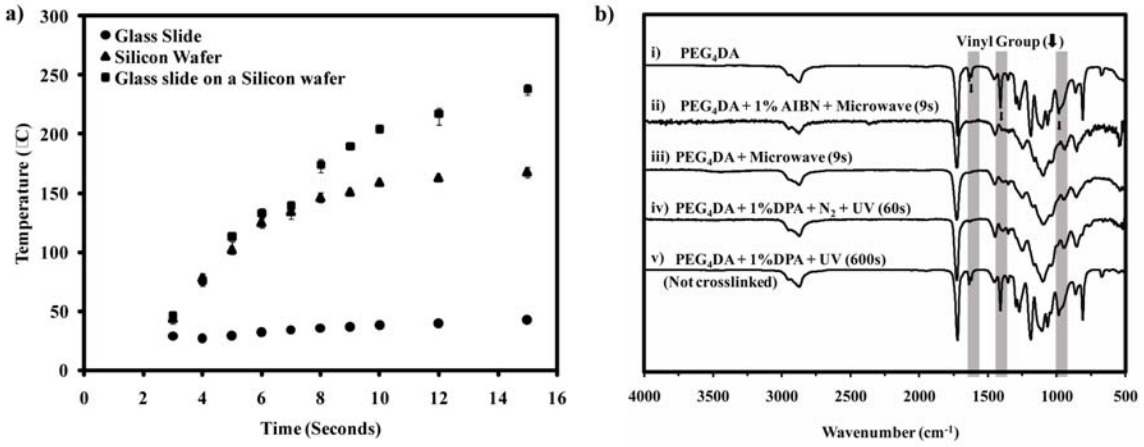

Figure 2. (a) Microwave-induced heating profiles of a glass slide (circle), a silicon wafer (triangle), and a glass slide on a silicon wafer (rectangular), respectively. (b) FTIR spectra before and after a crosslinking: (i) Uncrosslinked PEG_4DA monomer before microwave-induced heating, (ii) Crosslinked PEG_4DA polymer after microwave-induced heating with 1% AIBN for 9s, (iii) Crosslinked PEG_4DA under microwave-induced heating without 1% AIBN for 9s, (iv) Crosslinked PEG_4DA under UV irradiation (20 mW/cm^2) with 1% DPA for 60s in the presence of nitrogen, (v) Uncrosslinked PEG_4DA under UV irradiation with 1% DPA for 600s in the presence of ambient oxygen.

To directly compare the microwave-induced crosslinking with the UV-irradiated crosslinking, polymerizations were conducted under four different experimental conditions: (i) UV irradiation curing

without DPA photoinitiator for 600 s, (ii) UV irradiation curing with 1% DPA for 600 s, (iii) UV irradiation curing with 1% DPA for 600 s and then microwave-induced crosslinking for 9 s, and (iv) microwave-induced crosslinking for 9 s (Figure 3a). We selected these conditions to compare and quantify UV-irradiated and microwave-induced polymerization with and without the addition of exogenous DPA. To further compare the crosslinking methods, we used a method to quantify the amount of polymer that remained on the substrate after polymerization and washing steps. This was determined by using the ratio of the projected microstructure areas (RPA) which can be defined as the ratio of the area of a printed polymer spot (PA_0) to the area of the crosslinked polymer spot (PA_1) after washing (see S.I. Figure S4a). This ratio was calculated by quantifying the phase contrast images using the Image J software (see S.I. Figure S4b).[23] As shown in Figure 3ai and 3aii, UV-induced crosslinking required the addition of a free radical source. On the other hand, crosslinking under microwave irradiation rapidly progressed without an additional radical source at atmospheric condition. Furthermore, the time required for crosslinking was significantly reduced. Interestingly, we found out that a removal of the radical initiator did not inhibit the crosslinking reaction, providing an additional benefit for the use of microwave-assisted crosslinking.

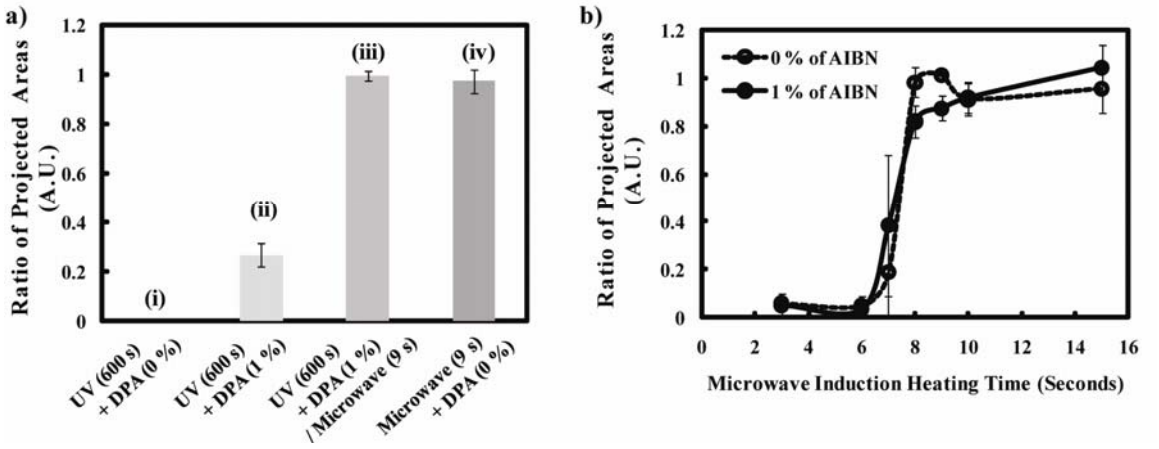

Figure 3. Polymerization of PEG_4DA under four different crosslinking conditions: (i) UV irradiation (20 mW/cm^2) without DPA for 600 s, (ii) UV irradiation with 1% DPA for 600 s, (iii) UV irradiation

with 1% DPA for 600 s and then exposure to microwaves for 9 s, and (iv) microwave-induced heating for 9 s. (a) Quantification of ratio of the projected areas. (b) Analysis of ratio of projected areas of PEG_4DA crosslinked by microwave-induced heating with and without 1% AIBN.

To further characterize the ability of the crosslinking reaction to continue without an exogenous source of initiator, we characterized the microwave-induced crosslinking of PEG_4DA with and without 1% w/v AIBN thermal initiator (Figure 3b). Recently, an initiator-free controlled polymerization under microwave irradiation was recently shown for polymerization of methyl methacrylate (MMA) at high temperatures.[24] In our experiments, both polymerizations with and without AIBN under microwave irradiation were completed within 10 s. This result suggests that PEG_4DA monomer stocks may contain impurities which initiate the reaction. In general, conventional PEGDAs and PEGDMAs are stored with various retarders such as hydroquinone (HQ), hydroquinone monomethyl ether (MEHQ), and butylated hydroxytoluene (BHT).[25] This is because increase of propagation rate by free radicals would result in unwanted polymerization.[26] To demonstrate performance of microwave irradiation for the rapid polymerization, we compared with UV irradiation curing methods using conventional PEG_4DA solutions. In our experiments, we found that these solutions were not polymerized during UV irradiation in air. In contrast, the microwave-induced heating approach induced rapid polymerization of these solutions under the same condition. These results indicate that microwave-induced crosslinking can be used to form microstructures without an additional radical source in the presence of radical impurities and retarders in air. Interestingly, the degree of polymerizations were similar for both cases, suggesting that the microwave-assisted polymerization was carried out regardless of the AIBN thermal initiator for a low volume associated with each printed polymer spot. The mechanism of the microwave-assisted PEGDA thermal crosslinking may be due to the thermal polymerization initiated by free radical species such as the radical impurities, peroxides, and oxygen plasma.

To further analyze this mechanism as well as to verify the reproducibility of the microwave-induced polymerization, we fabricated microwell structures by using a micromolding technique. First, we created microwells of PEG_4DA with and without AIBN (see S.I. Figure S5). The polymerizations with and without AIBN could be used to fabricate PEG_4DA microwells in 12 s and 9 s, respectively. Therefore, the polymerization rate for creating microwell structures was enhanced by radical flux from the decomposition of AIBN. These results imply that for a smaller reaction volume, e.g. a spot on a microarray, an addition of AIBN will not enhance polymerization rate, while for larger reaction volumes an addition of AIBN will enhance the reaction rate.

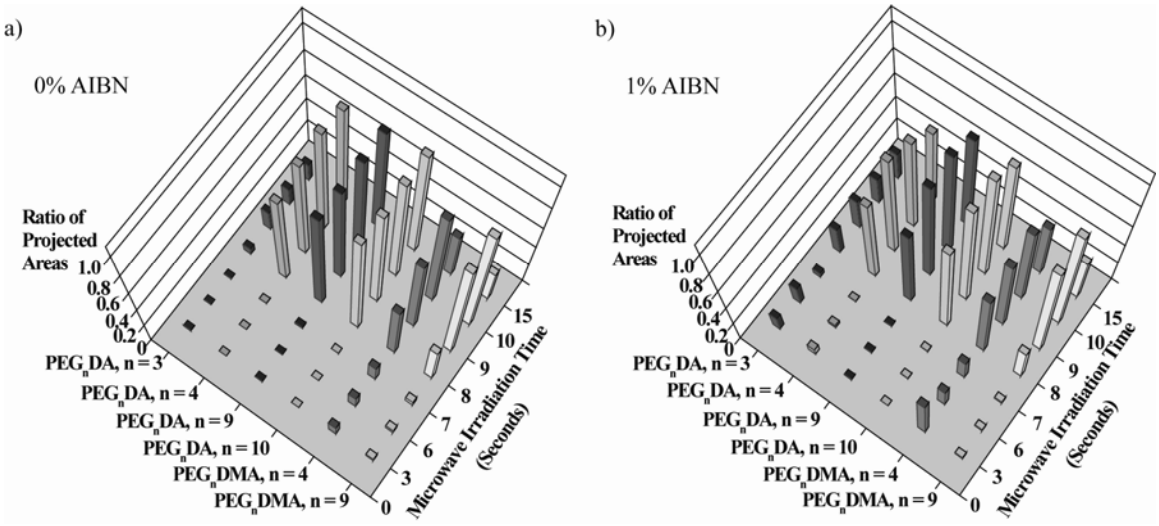

Figure 4. Analysis of crosslinked PEG-based acrylate polymers by using microwave-induced heating. Ratio of projected areas for various PEG_nDA (n = 3, 4, 9, 10) and PEG_nDMA (n = 4, 9) microarrays with (a) 0% and (b) 1% AIBN.

To validate that microwave-induced crosslinking can be used with a wide range of acrylated precursors, we crosslinked microarrays containing a range of PEG-based diacrylates and dimethacrylates, using the microwave-induced heating technique (Figure 4 and see S.I. Figure S6). All the acrylate monomers with and without 1% AIBN were consecutively printed by the robotic dispenser on the TMSPMA-coated

glass slides, followed by microwave-induced crosslinking and quantitative analysis of the ratio of the projected areas. All acrylate monomers with and without 1% AIBN started to be cured in 6 s and then were completely crosslinked in 9 s. We observed that after the printing, the area of the printed spots of PEG_4DA was larger than that of $PEG_{10}DA$. This is due to different levels of hydrophobicity of the acrylated monomers. Controlling the difference leads to controlling the spot areas after printing. On the other hand, the area of the polymerized PEG_3DA spot was reduced compared to that of the printed spot. This instability is caused by evaporation or shrinkage of the acrylate polymer under microwave irradiation.[8] Similar trends were also observed at larger exposure times. Therefore, the polymerization was optimized in 9 s for minimizing instability. To precisely manipulate the polymerization in a controlled manner,[27] future work will focus on synthesis of polymer microarrays using a microwave instrument containing an individual power controller and single-mode microwave reactor.

Conclusion

In this paper, we developed the microwave-induced crosslinking technique that enabled rapid and highly efficient polymerization of PEG-based acrylate monomers in the presence of ambient oxygen. In comparison to conventional UV-irradiated polymerization and thermal curing techniques, there are main advantages of polymerization by using this microwave-induced heating; (i) within short reaction time (<10 s), (ii) without an additional radical source (e.g., AIBN), and (iii) without oxygen inhibition in the presence of ambient oxygen. Furthermore, the results of FTIR spectra and the ratio of projected areas showed that the microwave-induced crosslinking did not cause thermal degradation. The results also imply that this technique is potentially beneficial to directly synthesize homogeneous PEG-based polymer arrays and microwells without prepolymerizations, solvents, and exogenous initiators. Therefore, this approach can be useful for various chemical and biological applications such as high-throughput screening of polymer libraries, cell-based biosensors, and biomedical microdevices.

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Keywords: microwave-induced heating; ultraviolet (UV) irradiation curing; polymer microarray; poly(ethylene) glycol diacrylate (PEGDA); ratio of projected area (RPA)

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Supporting Information for

Rapid formation of acrylated microstructures by microwave-induced thermal crosslinking

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Experimental Part

Materials

Poly(ethylene glycol)$_n$ diacrylates (PEG$_n$DA) (n = 3, 4, 9, 10) and PEG$_n$ dimethacrylates (PEG$_n$DMA) (n = 4, 9) were individually purchased from Polysciences, Scientific Polymer Products, and Sigma-Aldrich. 3-(trimethoxysilyl)propyl methacrylate (TMSPMA), 2,2´-azobisisobutyronitrile (AIBN) and 2,2-dimethoxy-2-phenyl acetophenone (DPA) were purchased from Sigma-Aldrich. Microscope glass slides (75×25 mm^2) were purchased from Fisher Scientific. Poly(dimethylsiloxane) (PDMS) molds were fabricated by curing prepolymer (Sylgard 184, Essex Chemical) on silicon masters patterned with SU-8 photoresist.

Characterization

Structures of PEG_4DA monomers and polymers were analyzed by using the conventional Fourier transform infrared spectroscopy (FTIR) spectrometer (Bruker ALPHA) with OPUS software. FTIR spectra were measured in an attenuated total reflection (ATR) mode. The data represent the average of 24 scans in the region between 4000 and 500 cm^{-1} at a resolution of 2 cm^{-1}. Samples were prepared after microwave-induced heating, conventional thermal heating and UV irradiation. After preparation of polymerization, all phase contrast images (10× and 2×) were taken on an inverted biological microscope (Nikon Eclipse Ti-S, USA) with SPOT advanced software. Representative image of a crosslinked PEG_4DA microspots in Figure S1 was taken by scanning electron microscope (SEM) (Carl Zeiss, ULTRA 55, Germany). The ratio of the projected area for each spot was calculated by analyzing phase contrast images using Image J software. The temperature of silicon wafer and glass slide after microwave irradiation was measured by using an infrared (IR) thermometer (OAKTON, WD35629, USA) at 23°C.

General procedure of TMSPMA-coated glass slide preparation

The glass slide was initially coated with TMSPMA, which provided an anchor for acrylate macromers during polymerization. Glass slides were cleaned with piranha solution (H_2O_2 : H_2SO_4 = 3 : 7) for 1h and washed 3 times with distilled deionized water (DDW) and 99% ethanol. 140 glass slides were stacked in a sealed box and coated with 4 mL of TMSPMA at 80°C for 24 h. Subsequently, the glass slides were washed 3 times with ethanol to remove excess TMSPMA, and cured in an oven (120°C) for 1h.

Microarray Preparation

Fresh stock solutions (1 mL) were prepared with neat acrylate monomers (1 mL) with and without a 1% (w/v) AIBN (10 mg). The solutions (100 μL) were immediately placed in 96-well plates. Microprinting was carried out by using a MicroGrid 600 robotic microarrayer (MGII600, Genomic Solutions, Harvard

Bioscience, USA). The acrylate monomers were printed using the arrayer with the MicroSpot 10k pin on the TMSPMA-coated glass slides under atmospheric conditions at 23 °C. To account for the different viscosities of the acrylated macromers, various aspects of printing such as pin washing time, pin speed, and pin priming were controlled. After printing, the acrylate monomers were crosslinked by using microwave or UV irradiation.

To crosslink the PEG acrylate microarrays by using microwave-induced heating, a silicon wafer (5.5 × 5.5 cm^2) was placed inside household domestic microwave oven (120 V_{ac}, 60 Hz, 1.3 kW, Avanti products). Uncrosslinked acrylate-printed glass slides were then placed on the silicon wafer and cured for specific durations. For UV irradiation curing, OmniCure®S1000 was used for a long wave UV irradiation (20 mW/cm^2) for 600 s. The distance between sample glass slide and UV resource was 15 cm. To fabricate PEG-microwells, uncrosslinked PEG_4DA (10 μL) was molded in the void space between PDMS mold patterned with microscale posts and a TMSPMA-coated glass slide. After curing, all glass slides were cooled and washed with DDW.

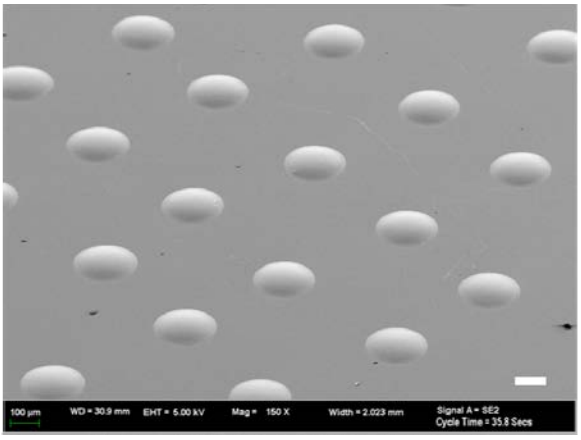

Figure S1. SEM image of microspots of PEG_4DA crosslinked by using the microwave-induced thermal crosslinking. Scale bar is 100 µm.

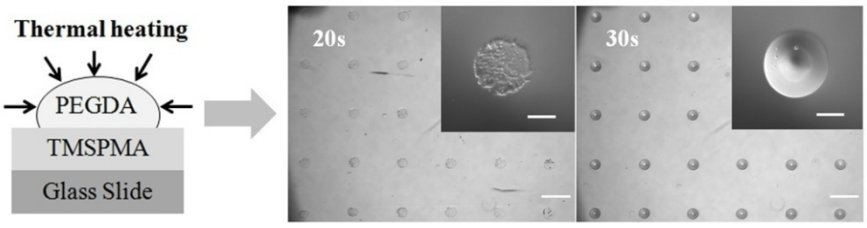

Figure S2. Schematic and phase contrast images of polymerized PEG_4DA by thermal curing (190°C) with 1 % AIBN for 20 s and 30 s. Scale bars are 100 μm (top inset) and 500 μm.

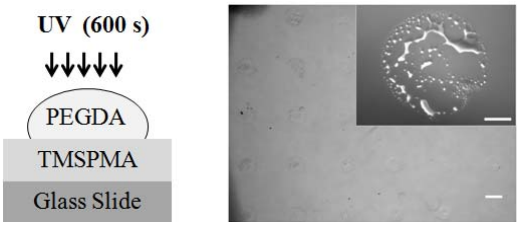

Figure S3. Schematic and phase contrast images of polymerized PEG_4DA microarray by UV irradiation curing for 600 s. Scale bars are 100 μm (top inset) and 300 μm.

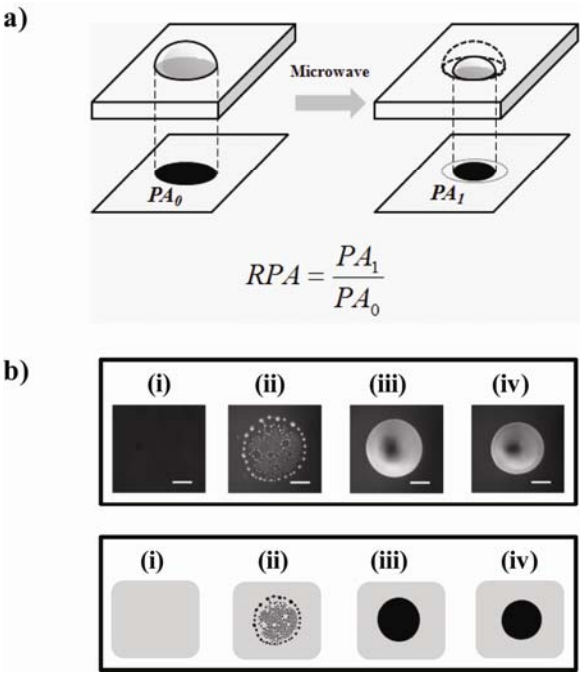

Figure S4. Polymerization of PEG_4DA under the four different crosslinking conditions: (i) UV irradiation (20 mW/cm^2) without DPA for 600 s, (ii) UV irradiation with 1% DPA for 600 s, (iii) UV irradiation with 1% DPA for 600 s and then exposure to microwaves for 9 s, and (iv) microwave-induced heating for 9 s. (a) Ratio of projected areas (*RPA*) induced with an initial projected area (PA_0) and a projected area after crosslinking (PA_1). (b) Phase contrast images of crosslinked PEG_4DA microspots in the individual polymerization and projected areas of the phase contrast images analyzed by Image J software. Scale bar is 100 μm.

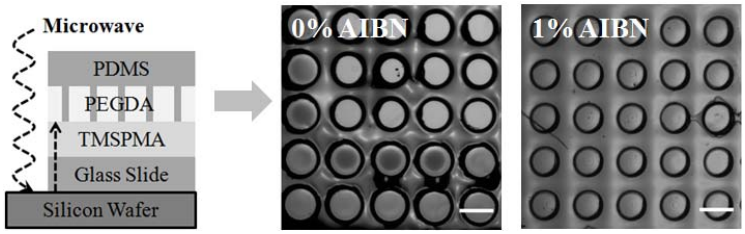

Figure S5. Schematic and phase contrast images of polymerized PEG_4DA microwells by microwave-induced crosslinking with and without 1% AIBN for 12 s and 9 s, respectively. Scale bars are 500 μm.

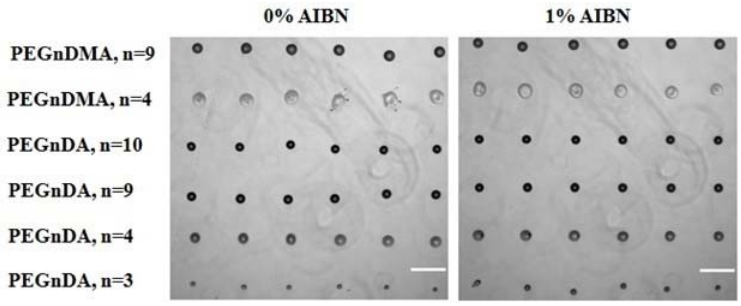

Figure S6. Phase contrast images of crosslinked PEG-based acrylate monomers by using microwave-induced heating with and without 1% AIBN for 9 s. Scale bar is 500 μm.

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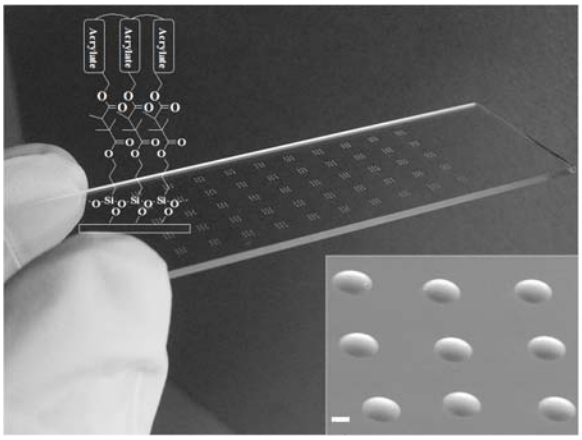

Poly(ethylene glycol)-based polymeric microarray was synthesized by using microwave-induced thermal crosslinking without exogenous radical initiator in air. The advantages of this process make it a potentially useful method for fabricating polymeric microstructures for a wide range of applications.